Relationships between Plasma Insulin-like Growth Factor-I and Insulin-like Growth Factor Binding Protein-3 and Second Breast Cancer Risk in a Prevention Trial of Fenretinide

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ABSTRACT

Purpose: High circulating insulin-like growth factor (IGF) -I and/or low IGF-binding protein (IGFBP) -3 levels are associated with increased breast cancer risk in unaffected premenopausal women. We determined whether IGF-I and IGFBP-3 predict second breast cancer risk, and whether their changes during fenretinide explain observed reductions in second breast cancer in women ≤50 years of age.

Experimental Design: Within a Phase III trial, we measured baseline and 1-year levels of IGF-I, IGFBP-3, and their ratio in 302 subjects on fenretinide and 220 controls who provided plasma samples. The prognostic effect of IGF-I and IGFBP-3, and the surrogate effect of IGF-I during fenretinide were assessed by Cox models after 9.4 years.

Results: Among controls, high IGF-I and low IGFBP-3 were associated with elevated second breast cancer risk [top versus bottom tertile, IGF-I, hazard ratio (HR) = 1.94, 95% confidence interval (CI), 0.87–4.31, P = 0.015; and IGFBP-3, HR = 0.40, 95% CI, 0.18–0.93, P = 0.033]. Fenretinide induced reductions of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 of 8% (95% CI, 2–12%; P = 0.004), 3% (95% CI, 1–5%; P = 0.002), and 5% (95% CI, 0–10%; P = 0.050), respectively. Second breast cancer risk was reduced by 39% (HR = 0.61; 95% CI, 0.40–0.94; P = 0.026). The percentage of treatment effect explained by IGF-I and IGFBP-3 reductions were 4.8% (95% CI, 0.8–28.9%) and 3.1% (95% CI, 0.5–20.8%), respectively.

Conclusions: Fenretinide induced a moderate reduction of IGF-I, which marginally explains observed cancer risk reductions in women ≤50 years of age. In this age group high IGF-I and particularly low IGFBP-3 levels predict second breast cancer risk.

INTRODUCTION

IGF-I2 is a key regulator of proliferation and apoptosis in normal and malignant cells, including breast cancer (1). Approximately 80% of circulating IGFs are bound to IGFBP-3, which affects cell growth by regulating binding of IGFs to the IGF receptors (2). In addition to modulating IGF-I and II, IGFBP-3 also has intrinsic inhibitory effects on breast cancer cell growth and survival that are independent of IGF-I (3, 4). A positive association between higher plasma IGF-I levels and/or lower levels of IGFBP-3 and risk of breast, prostate, colorectal, and lung cancers has been reported in large prospective studies in healthy people (5–9). For breast cancer, an association between high circulating levels of IGF-I and increased risk of breast cancer was found in premenopausal but not postmenopausal women (6, 10), consistent with the notion that IGF-I interacts with the estrogen signal to increase cell proliferation (11, 12). These findings suggest that lowering IGF-I availability may contribute to the reduction of breast cancer risk in premenopausal women.

Retinoids exert their antiproliferative effects in breast cancer cell lines partly by modulating the IGF system (13, 14). In a Phase III trial including nearly 3000 subjects with stage I breast cancer 30–70 years of age, treatment for 5 years with fenretinide, a synthetic derivative of retinoic acid, was associated with a 35% relative reduction in the incidence of contralateral breast cancer and ipsilateral breast tumor reappearance in premenopausal women (15). Importantly, previous studies had shown that fenretinide lowers plasma IGF-I levels differently depending on age or menopausal status, with a significantly greater reduction being found in women ≤50 years of age compared with older women (16, 17). Subsequent studies showed that the fenretinide-induced decline of IGF-I, IGFBP-3, and IGFBP-3 also has intrinsic inhibitory effects on breast cancer growth, partly by modulating the IGF system (13, 14). In a Phase III trial including nearly 3000 subjects with stage I breast cancer 30–70 years of age, treatment for 5 years with fenretinide, a synthetic derivative of retinoic acid, was associated with a 35% relative reduction in the incidence of contralateral breast cancer and ipsilateral breast tumor reappearance in premenopausal women (15). Importantly, previous studies had shown that fenretinide lowers plasma IGF-I levels differently depending on age or menopausal status, with a significantly greater reduction being found in women ≤50 years of age compared with older women (16, 17). Subsequent studies showed that the fenretinide-induced decline of IGF-I, IGFBP-3,

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2The abbreviations used are: IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; CI, confidence interval; HR, hazard ratio; ER, estrogen receptor.
and their molar ratio occurred during the first year of treatment and was maintained for up to 5 years of intervention, whereas no change was noted in IGF-II, IGFBP-1, and IGFBP-2 (18).

In contrast to healthy women, no data are available on the association of IGF-I and IGFBP-3 with the risk of second breast malignancy. Moreover, it is unknown whether a reduction in IGF-I levels during preventive intervention predicts the clinical effect of fenretinide and, therefore, will be a useful surrogate end point biomarker. The aims of the present study were to determine whether circulating IGF-I and IGFBP-3 levels are associated with risk of second breast malignancy in women ≤50 years of age and whether their changes during fenretinide treatment explain the beneficial effect on second breast malignancy observed in a Phase III trial (15).

MATERIALS AND METHODS

Subjects and Treatment. The present series was selected from a Phase III multicenter trial, of which the main results have been reported recently (15). Briefly, between March 1987 and July 1993, the trial enrolled 2867 assessable patients who met the following criteria: age between 30 and 70 years, breast cancer (T1-T2 or N0) or ductal carcinoma in situ treated with curative surgery within the previous 10 years, no adjuvant treatment, and no evidence of disease at study entry. Women were randomly assigned to receive no treatment or fenretinide (R.W. Johnson Pharmaceutical Research Institute, Springhouse, PA), 200 mg p.o. daily for 5 years (two capsules at dinner) with a monthly 3-day drug holiday. Measurements of IGF-I and IGFBP-3 were assessed on blood samples collected with the purpose of monitoring treatment compliance, as described previously (15). Specifically, samples had to be collected at randomization and after 1-year treatment only in women followed at the Istituto Nazionale Tumori, Milan, the coordinating center. After year 1, blood was collected on a yearly basis in all of the available subjects in the fenretinide arm and in a randomly selected group of subjects in the control arm (18).

To be eligible for the present study, patients had to fulfill the following criteria: (a) age ≤50 years at randomization; (b) available plasma aliquots both at baseline (pretreatment) and during follow-up; and (c) blood drawing within 24 h from last fenretinide intake. We defined the target population based on age ≤50 years instead of menopausal status consistent with our previous study (16), where age was a better modifier than was menopausal status of the effect of fenretinide administered for 1 year on IGF-I change. Blood aliquots were missing in 289 women because of insufficient blood amount or patient dropout (mostly because of nonmalignant adverse events during follow-up), thus leaving a total of 522 assessable subjects, 302 in the fenretinide arm and 220 in the control arm (Fig. 1). The randomly selected cohort of 60 subjects who were studied previously for the time profile of IGF-I and IGFBP-2 and IGFBP-1, -2, and -3 up to 5 years was included in the present analysis (18).

Assay Methods. Blood samples were drawn between 9 a.m. and 3 p.m. under variable fasting conditions, mostly (>80%) before lunchtime. Plasma aliquots obtained using heparin were separated by centrifugation and stored at −80°C until assayed. Plasma concentration of total IGF-I was determined by ELISA (3) kits purchased from Diagnostic Systems Laboratories, Inc. (Webster, TX). The sensitivity of the assay was 0.004 nmol/liter; intra and interassay coefficients of variation of our in-house pooled serum control sample were 4.9% and 7.5% (mean: 20.7 nmol/liter), respectively. To avoid interference by IGFBPs, the assay method was preceded by an acid/ethanol extraction procedure, as described previously (19). Serum IGFBP-3 was measured by ELISA using commercially available kits purchased from Diagnostic Systems Laboratories, Inc. The sensitivity of the assay was 0.001 nmol/liter; intra and interassay coefficients of variation of our in-house pooled serum control sample were 4.0% and 9.9% (mean: 222 nmol/liter), respectively. To include in the current analysis the series of 60 subjects (30 in each arm) in whom IGF-I and IGFBP-3 levels were measured by RIA and immunoradiometric assay, respectively, a calibration study between methods was performed to convert the values obtained in a previous study (18). For IGF-I, a total of 87 randomly selected subjects were run with RIA and ELISA. The calibration of IGF-I by Passing-Bablok regression (20) showed the following equation: $ELISA = 0.87 + 1.19*RIA$. Likewise, the calibration of IGFBP-3 obtained in 38 randomly selected subjects showed the following equation: $ELISA = 15.72 + 1.43*immunoradiometric assay$. All of the data were expressed in nanomolar concentrations. For IGF-I, to convert to μg/ml, one has to multiply by 7.633; for IGFBP-3, to convert to μg/ml, one has to divide by 24.

Statistical Methods. Measurements of IGF-I, IGFBP-3, and their ratio were log-transformed before analyses to approximate a Gaussian distribution. The geometric mean estimates and the 95% CI were calculated for baseline and follow-up biomarker levels.

Of the 522 subjects, 192 had two or more biomarker measurements during follow-up. The estimates of treatment effect on IGF-I and IGFBP-3 were obtained from linear regression models fitted on baseline and all of the follow-up measurements. The covariates were: treatment (no, yes), patient age at the time of blood drawing, and time from blood drawing to biomarker assessment to account for a possible decay of the
IGF-I or IGFBP-3 during the storage period. Age was non-linearly modeled (21), whereas a linear term was suitable for the decay interval. Correlation among different measurements within the same subject was accounted for by adopting a compound symmetry covariance matrix of residuals.

The study end point was the occurrence of a second breast malignancy, defined as ipsilateral breast tumor reappearance or contralateral breast cancer as first event. The two types of event were pooled before carrying out the analyses to increase the statistical power of the study, using an approach described previously by others (22). Furthermore, the protective effect of fenretinide proved to be similar on both events (15). All of the recorded events were included in the analysis, regardless of treatment duration and compliance levels, according to the intention to treat principle. Time to second breast malignancy was computed from the date of randomization, with censoring at the occurrence of progression to distant sites, second primary cancer in organs other than the breast, death with no evidence of disease, or the last follow-up available assessment.

Analyses of time to occurrence of second breast malignancy were performed by Cox regression models, checking the proportional hazard assumption by the graphical analysis of scaled Schoenfeld residuals. One model was fitted to assess the treatment effect in the selected sample of 522 women, with or without adjustment for menopausal status (pre- or postmenopausal), pathological tumor size (≤1.0 cm, 1.1–2.0 cm, or ≥2.1 cm), and histological type (infiltrating ductal carcinoma, infiltrating lobular carcinoma, infiltrating carcinoma with extensive intraductal component, or other).

The prognostic effect of baseline IGF-I, IGFBP-3, and their ratio was investigated by fitting Cox models only in the control group to avoid the interference of fenretinide treatment. Using an approach similar to Hankinson et al. (6), IGF-I and IGFBP-3 were entered into a model as categorical covariates by dummy variables, with classes defined by the respective tertiles. The same approach was adopted for IGF-I:IGFBP-3 molar ratio. For exploratory purposes, IGF-I and IGFBP-3 were also nonlinearly modeled as continuous covariates by restricted cubic splines with four knots (21). The analysis was not adjusted for ER expression of the primary tumor, because only 11.5% of the tumors had negative ER expression.

The analysis of surrogate end point biomarkers was performed according to the approach by Li et al. (23), by fitting for each marker a Cox model containing as covariates the marker itself, expressed as the ratio between the individual follow-up measurement and its baseline value, and treatment. When multiple follow-up measurements were available for the same subject, the ratio was computed after summarizing the repeated measurements by their median value or the 12-month (or closest) measurement. Because the two options led to similar results, we report those obtained with the latter. As required in the approach by Li et al. (23), the lack of a treatment by marker interaction was initially verified. The proportion of treatment effect explained by IGF-I or IGF-I:IGFBP-3 molar ratio was computed based on Cox model coefficients and the estimate of treatment effect on the biomarker levels obtained as described above. An ideal surrogate explains all of the treatment effect, where the proportion of treatment effect is equal to 1; however, surrogates explaining at least 50% of treatment effect can be considered adequate for practical purposes (24). After checking possible nonlinear effects (21), the biomarkers were entered into the Cox models by linear terms.

Ps reported are two-sided, and the conventional 5% significance level was adopted. The SAS software and the S-Plus Design library developed by Harrell (25) were used to perform the analyses.

RESULTS

Subject and Tumor Characteristics and Changes of IGFs During Intervention. The main characteristics of the 302 women in the fenretinide arm and the 220 women in the control arm (Table 1) were well balanced, with the exception of menopausal status (P = 0.025) and primary tumor stage (P = 0.023). In particular, slightly higher percentages of postmenopausal women and pT2 tumors were observed in the fenretinide arm. The study sample was comparable with the whole trial cohort (15); similarly, there was no significant difference in baseline characteristics with respect to the 289 subjects excluded from the analysis because of the lack of blood aliquots (data not shown). Although smoking habit was not systematically recorded in this clinical prevention trial, the proportion of current smokers was very limited in this affected cohort.

The median follow-up time was 9.4 years (9.5 years in the fenretinide arm and 9.1 years in the control arm). Overall, 83 breast malignant events were recorded, 30 ipsilateral breast tumor reappearances and 8 contralateral breast cancers in the fenretinide arm, and 31 ipsilateral breast tumor reappearances and 14 contralateral breast cancers in the control arm. The Cox
Table 2  IGFs geometric means (and 95% CIs, nmol/liter) by treatment group and reductions induced by treatment in women ≤50 years of age

<table>
<thead>
<tr>
<th></th>
<th>Fenretinide (n = 302)</th>
<th>Control (n = 220)</th>
</tr>
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<tbody>
<tr>
<td>IGF-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>17.6 (16.6, 18.8)</td>
<td>17.9 (16.5, 19.5)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>16.4 (15.3, 17.6)</td>
<td>17.6 (16.2, 19.0)</td>
</tr>
<tr>
<td>Reduction induced</td>
<td>7.6% (2.4%, 12.5%)</td>
<td></td>
</tr>
<tr>
<td>by treatment</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>IGFBP-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>156.0 (152.3, 159.8)</td>
<td>158.0 (154.1, 162.0)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>151.9 (148.4, 155.4)</td>
<td>155.5 (151.5, 159.7)</td>
</tr>
<tr>
<td>Reduction induced</td>
<td>2.9% (1.1%, 4.7%)</td>
<td></td>
</tr>
<tr>
<td>by treatment</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>IGF/IGFBP-3 ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.113 (0.107, 0.120)</td>
<td>0.113 (0.105, 0.122)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>0.108 (0.102, 0.115)</td>
<td>0.113 (0.105, 0.122)</td>
</tr>
<tr>
<td>Reduction induced</td>
<td>4.9% (0%, 9.5%)</td>
<td></td>
</tr>
<tr>
<td>by treatment</td>
<td>0.050</td>
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* Estimated by linear regression model adjusting for patient age and time from blood drawing to IGF assessment.

Table 3  Prognostic effect of baseline IGFs on risk of second breast cancer in control subjects (n = 220)

<table>
<thead>
<tr>
<th>Tertile classes*</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P*</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle vs. bottom</td>
<td>1.66</td>
<td>(0.77, 3.59)</td>
<td>0.200</td>
<td>0.239</td>
</tr>
<tr>
<td>Top vs. bottom</td>
<td>1.94</td>
<td>(0.87, 4.31)</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td>IGFBP-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle vs. bottom</td>
<td>0.67</td>
<td>(0.77, 3.59)</td>
<td>0.276</td>
<td>0.074</td>
</tr>
<tr>
<td>Top vs. bottom</td>
<td>0.40</td>
<td>(0.18, 0.93)</td>
<td>0.033</td>
<td></td>
</tr>
</tbody>
</table>

* Tertiles: IGF-I (15.2 and 23.6 nmol/liter), IGFBP-3 (156.4 and 192.0 nmol/liter).

* P at the Wald test for each comparison.

Overall P at the likelihood ratio test for testing the factor effect.

Fig. 2  Logarithm of the relative hazard (continuous line) and 95% confidence limits (dashed lines) of second breast cancer according to baseline IGF-I in control women. To convert to ng/ml, multiply by 7.633.

IGFBP-3 were stable in women <45 years of age and increased thereafter, although the trend was not statistically significant (P = 0.082). No significant decay of IGFBP-3 was noted (P = 0.512).

IGFs as Prognostic Factors of Second Breast Malignancy. High baseline circulating levels of IGF-I and low IGFBP-3 levels, when jointly modeled, were associated with the occurrence of a second breast malignancy in the control group (Table 3). When comparing the top versus bottom tertile class, the HR estimate was 1.94 (95% CI, 0.87–4.31; P = 0.11) for IGF-I and 0.40 (95% CI, 0.18–0.93; P = 0.03) for IGFBP-3.

The relationships between the risk of second breast malignancy and baseline IGF-I and IGFBP-3 as continuous variables are shown in Figs. 2 and 3. For IGF-I, a sigmoidal-like pattern was noted. The HR of the third versus first quartile, derived from the curve plotted in Fig. 2, was 1.61 (95% CI, 0.76–3.40). For IGFBP-3, a linear relationship was noted, with a HR for the interquartile range of 0.79 (95% CI, 0.34–1.84).

No significant association between IGF-I:IGFBP-3 ratio and second breast cancer was observed. When comparing top versus bottom tertile class, the HR estimate for IGF-I:IGFBP-3 was 1.30 (95% CI, 0.61–2.11; P = 0.50).

model including no surrogate biomarker showed a statistically significant (P = 0.026) 39% relative reduction in the occurrence of second breast malignancy in the fenretinide group compared with the control group (HR = 0.61; 95% CI, 0.40–0.94). A similar effect was estimated after adjustment for menopausal status, tumor size, and histological type (HR = 0.64; 95% CI, 0.41–1.01). These figures closely resemble the results observed in the original trial cohort of 1350 women ≤50 years of age (15), thus providing evidence against a possible selection bias in the present series. Other neoplastic events were progression to distant sites (33 in the fenretinide arm and 15 in the control arm) and second primary cancer in organs other than the breast (4 events in each arm).

Table 2 shows the mean baseline and on-study values of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio in the two treatment arms, and the reductions induced by treatment. There were no differences at baseline between arms. During follow-up, IGF-I and IGFBP-3 levels were lower in the treated group as fenretinide induced a significant reduction of IGF-I, IGFBP-3, and IGF-I:IGFBP-3 ratio by 8%, 3%, and 5%, respectively (Table 2). As expected, IGF-I was associated positively with IGFBP-3 both in the fenretinide group (Spearman correlation coefficient ρ = 0.45 for baseline and ρ = 0.44 for follow-up measurements) and the control group (ρ = 0.47 at baseline and ρ = 0.37 at follow-up measurements).

The estimates of the intrindividual variability and the within-subject correlation of IGF-I were 0.36 (on the logarithmic scale) and 0.61, respectively. The above values were not meaningfully different between arms. Notably, the intrindividual variability was more than four times the average reduction of IGF-I induced by fenretinide.

The levels of IGF-I declined significantly with age, more steeply starting from age 45 (P = 0.001; data not shown). Also, a significant decay of IGF-I was observed (P < 0.001), accounting for a 3% reduction per year of storage. The levels of IGFBP-3 were stable in women <45 years of age and increased thereafter, although the trend was not statistically significant (P = 0.082). No significant decay of IGFBP-3 was noted (P = 0.512).
Effect of IGFs as Surrogate Biomarkers of Fenretinide Effect. The results of different Cox models obtained with the inclusion of putative surrogate biomarkers are shown in Table 4. IGFBP-3 was excluded from this analysis based on its inverse association with second breast cancer risk coupled with its down-regulation during fenretinide administration. The concomitance of two counteracting trends represents an a priori characteristic to exclude IGFBP-3 as a potential surrogate end point biomarker. The proportion of treatment effect that was accounted for by the change in IGF-I and IGF-I:IGFBP-3 molar ratio was marginal. The highest proportion of treatment effect estimate was obtained for IGF-I (4.8%; 95% CI, 0.8–28.9%); thus, even considering the upper confidence limit, the 50% threshold of clinical relevance was not reached.

DISCUSSION

The high costs that are inherent in Phase III cancer prevention trials and the observation of unexpected detrimental effects of putative chemopreventive agents (26) have caused much emphasis to be placed on the search for intermediate, surrogate end points of intervention efficacy. These are defined as biological markers or events that may be assessed or observed before the clinical appearance of the disease and that bear some relationship to the development of that disease. Assessing the contribution of surrogate biomarkers to the clinical effect of intervention may not only increase efficiency of future clinical studies but provide clues into critical pathways associated with carcinogenesis and additionally characterize the activity of investigational agents (27).

Recent studies have suggested that circulating IGF-I and IGFBP-3 levels may qualify as ones of these biomarkers (6, 16). There is growing evidence that IGF-I is involved in the development of normal breast tissue (1, 11, 28) and may be associated with breast cancer formation in premenopausal but not postmenopausal women (6, 10). This age-related preferential effect is thought to be the result of an interaction between IGF-I and ER-mediated signaling that regulates cell proliferation in the normal breast gland, where estrogens sensitize target cells to the mitogenic effect of IGF-I (11, 12). In MCF-7 cells, the IGF-I receptor and the ER are coexpressed, and the two signaling systems are engaged in a cross-talk that results in synergistic growth (29, 30). Thus, women with elevated IGF-I bioactivity may be more susceptible to carcinogenesis stimuli given their increased epithelial cell renewal dynamics (1). Regarding IGFBP-3, recent data indicate that this protein has direct anti-proliferative/apoptotic effects on breast cancer cell lines that are independent of the regulation of IGF-I bioactivity (3). In addition, recent prospective studies have reported a statistically significant, inverse association between IGFBP-3 levels and risk of colorectal and lung cancers, whereas IGF-I showed no such association (8, 9).

Our preliminary studies (16, 17) had shown that the modulation of circulating IGF-I levels by fenretinide administered for 1 year differed according to age, with a statistically significant reduction only in women ≤50 years of age. This pattern is consistent with the clinical results of fenretinide, in which a statistically significant benefit on second breast cancer was shown only in premenopausal women or women who were ≤50 years of age (15).

To assess the plausibility of the associations between the preventive activity of fenretinide and its effect on the IGF system, we studied in a controlled trial setting whether IGF-I and/or IGFBP-3 levels predicted the risk of second breast cancer in the untreated control group, and whether the decline in circulating IGF-I, IGFBP-3, and IGF-I:IGFBP-3 during fenretinide treatment explained the clinical effects observed in women ≤50 years of age (15).

Our results show that women with high IGF-I and particularly those with low IGFBP-3 levels at baseline had a higher risk of developing a second breast malignancy. Fenretinide induced a moderate but statistically significant decline in IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio. However, the reductions of IGF-I and IGF-I:IGFBP-3 only marginally explained the clinical effect of fenretinide.

The findings of a higher risk of second breast cancer depending on high IGF-I and low IGFBP-3 levels at baseline are new and provide additional insight into the effect of the IGF system in premenopausal breast carcinogenesis. Our data indicate that a 35% difference in IGF-I levels between top and bottom tertiles is associated with an adjusted HR of 1.94 (95% CI, 0.87–4.31) of developing second breast cancer. The association with IGFBP-3 was even stronger. The adjusted HR of 0.40 (95% CI, 0.18–0.93), associated with a 20% difference in IGFBP-3 tertile levels, is in keeping with the emerging epidemiological evidence of an independent effect of this protein on cancer development (8, 9, 31). Notably, the positive association

![Fig. 3 Logarithm of the relative hazard (continuous line) and 95% confidence limits (dashed lines) of second breast cancer according to baseline IGFBP-3 in control women. To convert to μg/ml, divide by 24.](image-url)
between baseline IGF-I and IGFBP-3, together with our findings of their opposite effect on second breast cancer risk, underline the importance of modeling jointly the two biomarkers to estimate their independent effect. In the Nurses’ Health Study (6), a 24% difference in IGF-I levels between top and bottom tertiles was associated with a significantly increased risk of breast cancer in premenopausal women (relative risk = 2.33, 95% CI, 1.06–5.16). The relative risk increased to 7.28 (95% CI, 2.40–22.0) when the analysis was restricted to premenopausal women ≤50 years of age and after adjustment for IGFBP-3. In the study by Toniolo et al. (10) in premenopausal women ≤50 years of age, a 35% difference in IGF-I quartiles was associated with an odds ratio of 2.30 (95% CI, 1.07–4.94), which slightly decreased after adjustment for IGFBP-3 (odds ratio = 1.90; 95% CI, 0.82–4.42). The two studies did not describe the adjusted effect of IGFBP-3 on the risk of developing breast cancer. However, Hankinson et al. (6) reported a nonsignificant inverse association, whereas Toniolo et al. (10) found no significant direct association between IGFBP-3 and risk of breast cancer. Altogether, the findings point to a crucial role of the IGF system in the development and progression of breast cancer in premenopausal women.

Interestingly, in our study the pattern of risk according to IGF-I levels appears to follow a sigmoidal dose-response curve, thus suggesting a receptor saturation kinetics model. At variance, the quasi-linear effect of IGFBP-3 on second breast cancer risk suggests a different mechanism of action. Indeed, a signaling receptor for IGFBP-3 has not been characterized thus far, and IGFBP-3-induced growth inhibition and apoptosis do not require cell surface binding and nuclear translocation in breast cancer cell lines (32).

Fenretinide induced a statistically significant decline of 8%, 3%, and 5% in IGF-I, IGFBP-3, and IGF-I:IGFBP-3 molar ratio, respectively. The reduction in IGF-I was slightly lower than those reported by us in previous studies (16–18). Importantly, the reduction of IGF-I was less than a quarter within subject variability (SD = 36%). One explanation for such a relatively high variability might be the different conditions of blood drawing, in which time, fasting, and menstrual cycle could not tightly be controlled in the present study. A few studies have shown that IGF-I measurements are fairly stable over 24 h (2), whereas several days of fasting are known to decrease IGF-I levels (33). However, no difference in IGF-I and IGFBP-3 was noted in a recent study between women who were fasting and those who had breakfast (34). In our study, the vast majority of blood drawings were obtained before lunch under variable fasting conditions. Another potential source of variability is the menstrual cycle, which has been associated with IGF levels with conflicting results. Although most studies showed no association (35), a recent study indicated that IGF-I:IGFBP-3 levels were lowest during the early follicular phase, and rose steadily during the follicular and luteal phase, peaking just before menses (34). Although IGF-I levels are known to be extremely variable among subjects, with genetic factors explaining ~50% of the interindividual variability (34), our study indicates that also intranidividual variability is high.

The finding of a mild decrease in IGFBP-3 during fenretinide treatment confirm our recent observation in a random subset of the trial cohort (18). The results vary from our initial finding of a slight increase of IGFBP-3 levels in a small group belonging to the same trial (17), possibly as a consequence of the limited sample size in our initial observation. In a different context, i.e., men with superficial bladder cancer treated with fenretinide, no change in IGFBP-3 levels was observed (36). The reason for these discrepancies is unclear, although they may at least in part be attributable to differences in genetic and lifestyle factors among the populations studied. For instance, a single nucleotide polymorphism in the promoter region of IGFBP-3 has been shown to influence the effect of retinoids on IGFBP-3 expression (37). In addition, retinol levels have been shown to positively influence IGFBP-3 levels in women carrying an A allele at −202 in the promoter region of IGFBP-3 (37). Likewise, oral contraceptive use (34), tallness and overweight (37), and smoking habit (38) have been shown to influence IGFBP-3 concentrations. Although in the present study the influence of these factors could not be determined, additional studies should consider their role as potential sources of variability.

An important finding of our study is the observation that the reduction of IGF-I explained only ~5% of the clinical effect of fenretinide, a level ~10-fold lower than the 50% threshold of clinical relevance (24). Notably, IGFBP-3 could not be considered as a suitable surrogate end point biomarker given its decline while on fenretinide coupled with its inverse association with breast cancer risk. These results seem to suggest that inhibition of circulating IGF-I bioactivity is not an important pathway of the preventive effect of fenretinide on second breast cancer that is observed in women ≤50 years of age. However, both the moderate reductions of IGF-I during fenretinide treatment and the large variability within subjects may well have diminished the explanatory power of IGF-I as a surrogate biomarker of the clinical effect of the retinoid.

Because only 522 (39%) of the 1350 women ≤50 years of age who participated in the Phase III trial were included in the present study, there are potential limitations in terms of selection bias and low statistical power. For instance, a slight imbalance in menopausal status and tumor stage was noted between the two groups. However, the subject characteristics and the clinical effect of fenretinide in this study group were very similar to those observed in the overall cohort (15) and in the subset of 289 patients excluded from the analysis because of missing blood aliquots. The above-mentioned imbalance did not substantially influence treatment effect estimates, which were similar in adjusted and unadjusted analyses. Likewise, the available sample size was sufficient to confirm the statistically significant effect of fenretinide on second breast cancer and, based on the upper confidence limit of the proportion of treatment effect explained by IGF-I, proved to be adequate to exclude a relevant surrogate effect of the biomarker. Nevertheless, because our original chemoprevention trial was designed to look at clinical, not laboratory, end points, our results may even be considered hypothesis generating and should be confirmed in future studies.

In conclusion, our study shows that the moderate modulation of IGF-I by fenretinide is not an important pathway of the clinical effect of the drug on second breast cancer. However, the association between baseline IGF-I and particularly IGFBP-3 level and subsequent breast cancer suggests that other strategies aimed at reducing circulating levels of IGF-I or increasing...
IGFBP-3 levels are a plausible way to reduce breast cancer risk in a primary as well as secondary prevention setting. Additional breast cancer intervention studies using different agents should incorporate IGF-I and IGFBP-3 to address these important issues.

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REFERENCES


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